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Ageing affects microplastic toxicity over time: Effects of aged polycarbonate on germination, growth, and oxidative stress of *Lepidium sativum*

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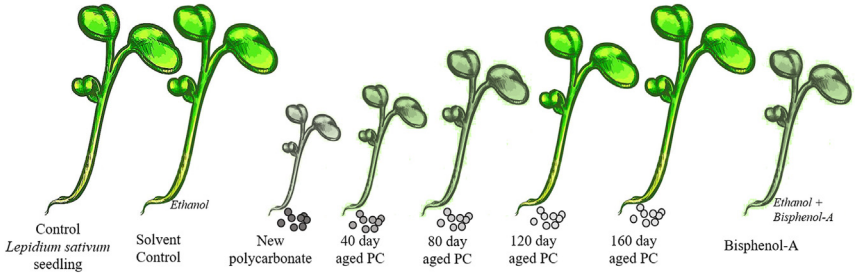
HIGHLIGHTS

- New and short-term aged PC cause *Lepidium sativum* sprouting and growth inhibition.
- *L. sativum* catalase activity increased with new and short-term aged PC exposure.
- PC as new and short-term aged granules reduced chlorophyll *a* and *b* concentrations.
- All adverse effects observed in *L. sativum* decreased with increased PC ageing time.

GRAPHICAL ABSTRACT



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ABSTRACT

Plastic has been an environmental pollutant far longer than claimed by the first reports surfacing in 1979, meaning some plastic materials have been decaying in nature for decades. Nevertheless, the threat posed to biota is not fully understood, especially from aged microplastic. The question considered in this study was whether the adverse effects of new plastic differ from those of old plastic material. Therefore, the morphological and physiological effects on *Lepidium sativum* with exposure to both new and aged polycarbonate were considered against a known stressor leaching from polycarbonate with time, bisphenol-A. Exposure to new and short-term aged polycarbonate (up to 80 days) elicited the most severe effects such as germination inhibition, reduced seedling growth, decreased chlorophyll concentrations, and increased catalase activity. These adverse effects in *L. sativum* associated with polycarbonate exposure were reduced as a function of the ageing time applied to the polycarbonate. The chemical substances that lend new polycarbonate material its toxicity were likely leached with time during the ageing process. Based on the results obtained, temperature and humidity based artificial ageing significantly reduced the phytotoxicity of the microplastic particles.

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1. Introduction

Plastic pollution is not a new concern as the first evidence of its environmental distribution were given in 1979 via a study on pelagic tar and plastic in the ocean (Shaw and Mapes, 1979), as well as the description of patches of plastic waste in the ocean described back in 1986 (Day, 1986). Thus, more than 40 years ago, this problem was acknowledged and began to arise, with low interest at first, despite some researchers and activists' efforts, among those Captain Charles Moore (Moore, 2008). However, in recent years, studies on the distribution (Isobe et al., 2019; Choy et al., 2019; Scopetani et al., 2019; Yakushev et al., 2021) and hazardous nature of microplastic (MP) threatening our environment (Desforges et al., 2015; Zimmermann et al., 2020) have dramatically increased, demanding the focus of science and considerable public attention (Horton et al., 2017).

Plastic debris accidentally released or intentionally discarded into our environment is technically defined as MP when the particle size is smaller than 5 mm (Koelmans, 2015). Larger pieces will undergo destruction and embrittlement mainly by weathering effects involving heat, UV-light, hydrolysis, and the major overall factor "time" (Hamid et al., 1992), eventually yielding MP. However, with time many factors come into play, such as chemical leaching and dissolution. To date, the fate of MP toxicity with time is poorly understood and seeing as MP pollution has existed for as long as plastics have been in production and is steadily increasing, it is essential to establish the environmental effects of aged MP.

MP is considered a global threat to our environment (Rochman et al., 2013) and present in all ecosystem compartments, including water (Isobe et al., 2019), soil (Rillig, 2012), and even air (Zhang et al., 2020). The direct effects of ingested MP were reported, e.g. for zebrafish (*Danio rerio*) (Lu et al., 2016), the grass shrimp (*Palaemonetes pugio*) (Gray and Weinstein, 2017), and the blue mussel (*Mytilus edulis*) (Browne et al., 2008), as well as zooplankton (Cole et al., 2013; Rehse et al., 2016). The transfer of MP between trophic levels has been hypothesised and also demonstrated (Farrell and Nelson, 2013; Setälä et al., 2014; Araújo et al., 2020; Araújo and Malafaia, 2021). Even the intake of MP particles by humans has been calculated, i.e. from drinking mineral water from plastic bottles, and estimated to be around 90,000 particles per year (Cox et al., 2019).

The presence of MP in soil (Piehl et al., 2018) and the subsequent effects on plant growth and physiology (Rillig, 2012; Rillig et al., 2019; Pflugmacher et al., 2020) has been hypothesised to strongly dependent on the MP types (e.g. polycarbonate (PC), polyethylene, polyvinyl carbonate, polystyrene etc.) and possibly shape (granule, fibre, fragments etc.). The effects have been considered to be negative as well as positive when considering MP as a carbon source for the soil microbial community, the changes MP causes in soil structure, influences on bulk density, water holding capacity, sorption of xenobiotics on the surface of MP particles, or even direct toxicity (Rillig et al., 2019). However, the effects of aged MP on plants remain without underlying experimental data (Rillig et al., 2019).

In the present study, it was hypothesised that the factor of time, or in the case of MP, ageing of the particles, will significantly influence the phytotoxicity due to the leaching of additives that possibly lend the toxic characteristics to MPs. The aim was to investigate possible adverse effects of artificially aged PC granulate (aged for varying periods) and bisphenol A (BPA) on seed germination, seedling growth, and physiological parameters such as pigment content and the antioxidative enzyme activity of catalase. *Lepidium sativum* (garden cress) was selected as the model organism in which to study these effects as it is often used in ecotoxicological studies related to adverse effects on terrestrial plants. Previous studies on the adverse effects of microplastic on *L. sativum* proved its applicability and laid the groundwork for this study (Bosker et al., 2019; Pflugmacher et al., 2020). PCs stand out among the plastics as they are one of the most widely produced and used engineering thermoplastics and find many applications in food packaging, the building industry, optics, electronics, vehicles and other industries due to their chemical properties (Liang et al., 2015). PC is also used in agriculture to produce greenhouses or growth tunnels due to its transparency to sunlight. However, under environmental conditions, PCs degrade with time (Ram et al., 1985), and end up in nature and was thus selected as test material in the present study.

2. Material and methods

2.1. Lepidium sativa (L.) seeds

Garden cress seeds (*Lepidium sativum* L.) (Art. No. G250) were purchased from Bingenheimer Saatgut AG (Echzell, Germany). The seeds were scrutinised, and any small, deformed or damaged seeds were removed. The seeds were washed in distilled water to remove possible impurities. The batch used for all experiments exhibited a germination percentage of 99 ± 1%. For all experiments, the plants were grown at 25 °C ± 1 °C under a light intensity of 30 μmol photons m^{-2} s^{-1} and a light-dark cycle of 12:12 h for seven days in total.

2.2. Microplastic artificial ageing

Commercially available transparent polycarbonate granulate (PC) with an average granule size of 3 mm ± 1 mm (Goodfellow GmbH, Hamburg, Germany) was used for the artificial ageing and subsequent exposure. The granulate diameter and shape were verified microscopically. The plastic granules were washed for a few minutes with ISO reconstituted water (pH 7.2) (ISO, 1996) to remove super fine particles, followed by drying at 25 °C.

Hygrothermal accelerated ageing of the PC material was performed following the method PN-EN 12280-1:2002 (Fejdyś et al., 2011). A thermal chamber (TK 720 Binder GmbH, Tuttlingen, Germany) was used to age the plastic with a heating effect. The temperature was set to 70 °C ± 0.5 °C and 1% ± 0.5% humidity. The PC granulate was aged in this chamber for 40, 80, 120, and 160 days, respectively.

Bisphenol A (BPA), a building block of PC previously reported to leach from the material (Carwile et al., 2009), was selected to elucidate the effects of substances possibly leaching from MP material on the germination and growth response of the garden cress, *L. sativum* after seven days. The period of seven days was selected as up to 88% of BPA was reported to dissipate in the soil after three days (Fent et al., 2003), adding an additional four days to observe the subsequent effects in the seedlings, totalling seven. BPA was dissolved in ethanol to a final solvent concentration of 50 mg L^{-1}, equivalent to 0.219 mM, and this solution was used for irrigation of the BPA treatment groups. The solvent concentration (ethanol) in the soil was 2%. A solvent control was set up in parallel to investigate the added effects of ethanol.

2.3. Plastic characterisation

Positive identification of the used polycarbonate plastic and the effect of ageing on the plastic was performed using PerkinElmer, Spectrum One (ATR-unit) for IR-spectra (Fourier-transform infrared spectroscopy, FTIR), using 8 scans with a resolution of 4 cm^{-1} in a range of 4000–650 cm^{-1} (Scopetani et al., 2019). Raman spectroscopy was applied using a Renishaw Invia Qontor confocal microscope at 785 nm, grating 1200 I mm^{-1}, exposure time 1 s, 30 accumulations with 100% laser power, centre 1300 Raman shift/cm^{-1} and a fifty times objective (Lee et al., 2000).

2.4. Experimental setup

Turf-free soil was purchased from MeinWoody (Grub am Forst, Germany) and consisted of 20% lingo fibres, 35% cocopeat washed, 10% spelt fermented, and 35% substrate compost. The soil had a pH of 5.85 ± 0.04.

In all experimental setups and during analyses, care was taken to avoid self-contamination (Scopetani et al., 2020), i.e. not wearing clothing or using lab equipment made of plastic that could contribute MP into the setup.

The effects of the aged PC and BPA on the germination of *L. sativum* were tested in 60 mL glass crystallising dishes (35 mm high by 60 mm diameter) containing 6 g of the commercial substrate (hereafter referred to as soil) in 30 replicates and ten seeds per replicate (in total, 300 seeds). The respective PC material (new and PC aged for various periods; 2% (w/w)) was carefully mixed into the soil until a visually homogeneous mixture was achieved. The concentration was selected based on the MP concentration ranging from 0.03% to 6.7% (w/w) reported in highly contaminated soils, which were related to the vicinity of sample sites to the industrial area (Fuller and Gautam, 2016). BPA treatments were irrigated with 0.5 mL of 50 mg L^{-1} BPA (25 μg BPA/6 g soil) over the course of the experiment amounting to 4166 μg kg^{-1} soil assuming homogenous distribution. The volume was added directly to the soil, where the seed was sown with a pipette.

The effects on the seedling growth parameters were evaluated using pre-germinated *L. sativum* seeds placing each seed singly in round-bottom test tubes made of soda glass (VWR, Germany) with a size of 100 × 16 × 1.0 mm filled with soil and PC mixtures, respectively. Three blocks of 50 seeds in three replicates in a total of 150 seeds were set up. The seeds were grown in a climate-controlled room with adjustable growth lights, i.e. at 25 °C ± 1 °C under a light intensity of 30 μmol photons m^{-2} s^{-1} and a light-dark cycle of 12:12 h for seven days in total. After measuring the seedlings' lengths and weighing as detailed below, the samples were snap-frozen in liquid nitrogen and stored at −80 °C until pigment or enzyme extraction was performed. Pre-germinated unexposed seeds were used to evaluate the effect on seedling growth only, without possible prior physiological damage due to exposure during germination.

2.5. Growth parameters analysis

2.5.1. Germination

Germinated seeds were defined operationally as having a radicle emergence length of 1 mm. The final germination percentage (GP) was determined after seven days, according to the following formula:

$$GP = \frac{Ng\ at\ day\ 7}{N\ t} \times 100$$

where Ng is the number of germinated seeds, and N*t* represents the total number of seeds used in the respective batch. The unit for GP is a percentage (%) (Janssen, 1973; Scott et al., 1984).

2.5.2. Seeding growth

The seedlings were carefully harvested from each tube after seven days, washed with distilled water to remove soil particles, and analysed for the various growth parameters. The growth response was measured in terms of the root, the shoot, and the overall seedlings length. Seedlings were cut at the root-shoot junction, and the roots and the shoots, respectively, were measured (in millimetre) with a digital calliper. Total length was calculated as the sum of both measurements (Chou and Lin, 1976).

The roots and shoots' fresh and dry weight were recorded on an analytical balance and expressed in mg after carefully drying the plant parts between two tissue papers. For dry weight, the plant sections were dried in an oven at 60 °C for 24 h until a constant dry mass was observed (Bush, 1995).

2.5.3. Pigment analysis

The pigment analysis (Chl *a* and Chl *b*) was performed on a plate reader (Tecan Infinite 200 PRO Nano+, Tecan Switzerland). Leaves of seven-day-old *L. sativum* seedlings were cut in half and frozen. The leaf material was ground to a fine powder and 0.1 g (FW) was mixed with 0.2 mL of *N*,*N*-dimethylformamide (DMF) and further homogenised on ice with a glass potter. The homogenate was centrifuged for 2 min at 5.000 ×*g*, and the supernatant was used for the analysis. The absorbance was measured at 647 (max Chl *b*) and 664.5 nm (max Chl *a*) against DMF as a blank. The pigment content was calculated using the equations given by Inskeep and Bloom (1985).

2.5.4. Catalase activity assay

Enzyme extracts of roots and shoots of *L. sativum* were prepared separately by grinding the tissues in liquid nitrogen and transferred the powder to a vial with sodium phosphate buffer (50 mM, pH 6.5), containing 0.2 mM EDTA. Additional homogenisation was performed using a glass homogeniser on ice. The samples were then centrifuged at 10,000 ×*g* for 10 min. The supernatant was used to determine the catalase enzyme activity. Catalase activity (CAT, E.C. 1.11.1.6) on the same plate reader was measured at 240 nm, with the decrease of absorbance correlated to the degradation of H_2O_2 (Aebi, 1984). The reaction mixture consisted of 50 mmol L^{-1} sodium phosphate buffer, 10 mmol L^{-1} H_2O_2 and 10 μL enzyme extract. Enzyme activity of CAT was defined as 1 mmol of H_2O_2 oxidised over 1 min at 25 °C and expressed in μkat mg^{-1} protein. Protein determination was performed, according to Bradford (1976).

2.5.5. Calculation of stress tolerance indices

The stress tolerance index for the different growth parameters was calculated as the growth of the roots, shoots or weight of the seedlings under stress (exposure to new or aged PC or BPA) as a percentage of the same parameter under non-stressed situations (controls), according to Wilkins (1957) as seen in the equation below.

$$Stress\ tolerance\ index = \frac{Root\ or\ shoot\ lenght\ or\ weight\ with\ exposure}{Root\ or\ shoot\ lenght\ or\ weight\ of\ controls} \times 100$$

2.6. Statistical analysis

IBM® SPSS® Statistics Version 25 (2018) was used to perform descriptive analyses based on the mean of the different endpoints chosen. After assessing the normality and homogeneity of the data, the non-parametric Kruskal Wallis test and pair-wise comparisons were used to identify statistically significant differences between treatment groups. The α-value considered for significance was 0.05 after Bonferroni correction (Sokal and Rohlf, 1997).

3. Results and discussion

3.1. Plastic characterisation

Following artificial ageing of the PC granules, the Fourier-transform infrared spectroscopy (FTIR) and Raman spectroscopic spectra showed no differences between the new PC and the aged PC, even with ageing up to 160 days (Fig. 1). The FTIR and Raman spectra exhibited PC corresponding fingerprints; i.e. the expected characteristic peaks for PC was observed (Shekhawat et al., 2011; Lee et al., 2000), and the monomer units (or structure) did not change with ageing, with minimal shifts observed in the frequencies indicating very low bond stretching or bending energy, i.e. subtle conformational changes in the polymer structure (Lee et al., 2000). Lee et al. (2000) previously showed that thermal ageing caused minimal wavenumber shifts. However, via the FTIR spectra, changes in the absorbance of the peaks were observed, indicating bond destruction, possibly by hydrolysis, which could release BPA (Deirram and Rahmat, 2012). Contamination with chemical impurities was not detected in any of the PC samples.

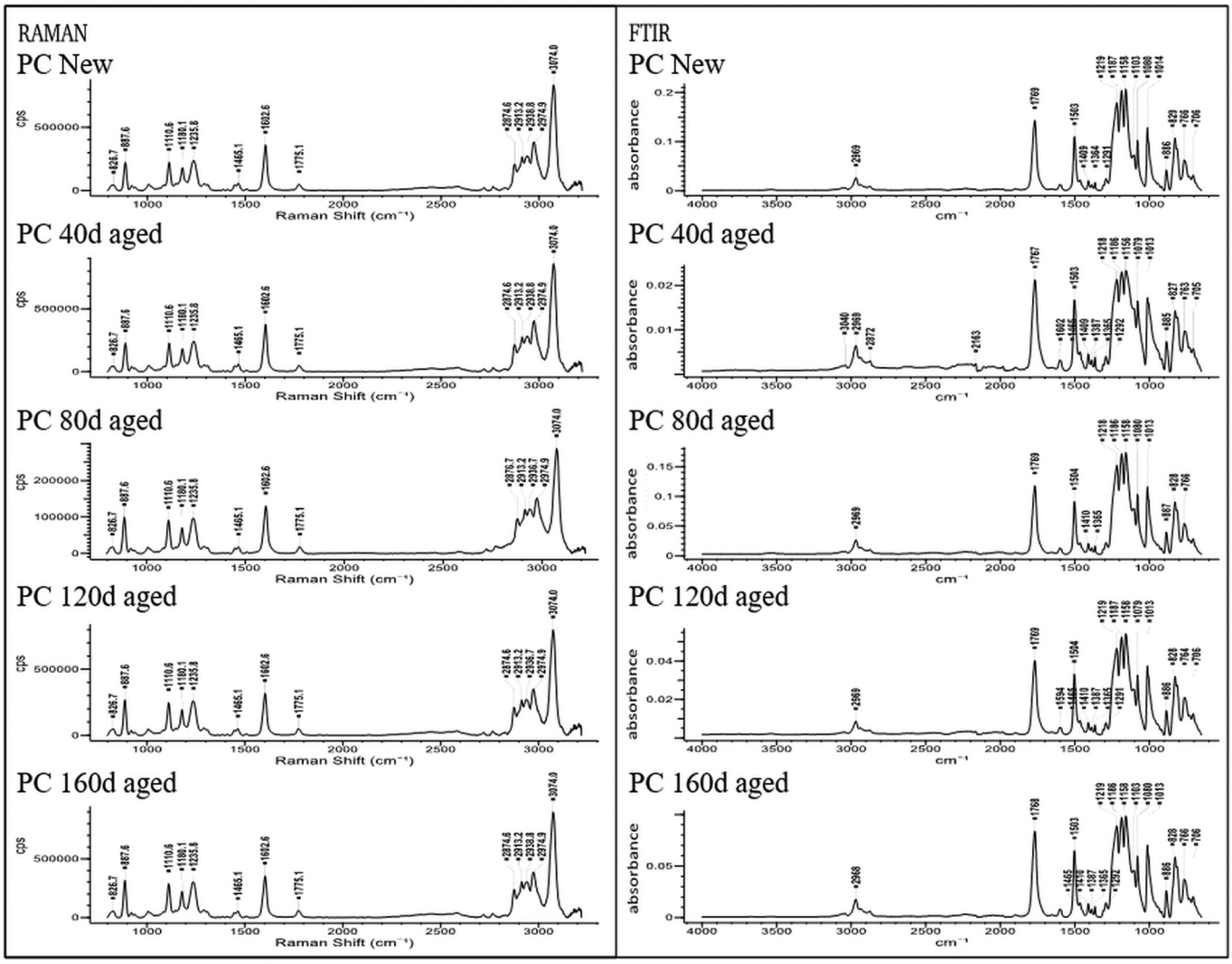

Fig. 1. Raman (left side) and Fourier-transform (FTIR) (right side) analyses of new and aged polycarbonate (PC) material. CPS indicates Raman intensity.

3.2. Germination

Based on the current MP concentrations reported in soils, the maximum reported in highly contaminated soils, and considering future projections (Fuller and Gautam, 2016; He et al., 2018; Meixner et al., 2020), an exposure concentration of 2% (w/w) PC granules was selected. Exposure to the new PC granulate in the soil reduced the germination percentage of the *L. sativum* seeds by 60% (Fig. 2) compared to control ($p < 0.001$). PC material aged for 40 days resulted in the same inhibition percentage (58%, $p = 1$). With extended ageing, reduced but still statistically significant inhibition was observed, decreasing to 46% for 80-day aged MP ($p < 0.001$), 16% inhibition for 120-day ($p = 0.010$), and 15% with exposure to 160 day-aged PC ($p = 0.020$).

During germination, imbibition especially refers to the absorption of water by the dry seed for rehydration. It is assumed, only substances solved in water could account for the inhibitory effect observed. It was previously shown that MP particles could block the pores in the seed capsule, thereby adversely affecting germination (Bosker et al., 2019). However, in the presented study, the PC particles were 3 ± 1 mm in diameter (compared to the 50, 500 and 4800 nm particles used by Bosker et al., 2019), making blockage an unlikely scenario. More likely, the effects were due to compounds leached from the granules. Interestingly, the germination inhibition percentage decreased as a function of the period for which the PC material was aged. The results suggest that more toxic substances are released from the plastic with extended ageing, which left less to leach out during the experimental exposure period and available to the *L. sativum* seeds.

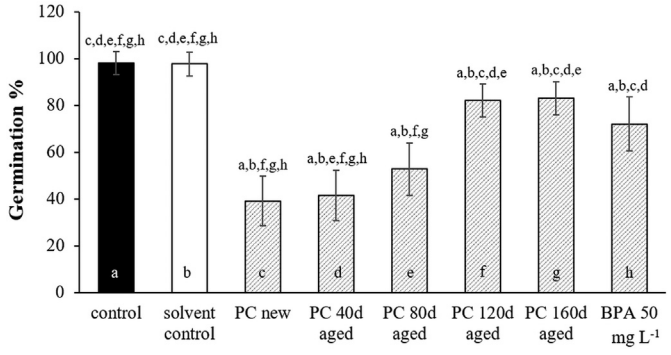

Fig. 2. Effect of new and aged (in days, d) polycarbonate (PC) microplastic derived from bottle caps and 50 mg L^{-1} bisphenol-A (BPA) on the germination of *L. sativum* after seven days. Data represent the average germination percentage ± standard deviation ($n = 400$). The Kruskal-Wallis ($H(7) = 208.8$, $p < 0.001$) test was performed, followed by pair-wise comparison. Statistical significance ($p < 0.05$) between groups is represented by the letters above the bars; i.e. a: compared to control; b: compare to solvent control; c: compared to PC new; d: compared to 40d; e: compared to 80d; f: 120d, g: 160d; and h: BPA.

BPA, an estrogenic agonist and androgenic antagonist, has previously been shown to evoke reproductive development issues in animals (Chapin et al., 2008). In plants, a low concentration of BPA (1.5 mg L^{-1}) was reported to promote soybean seedlings' growth hormones and growth. However, higher concentrations (3 to 96 mg L^{-1}) resulted in inhibitory growth effects (Wang et al., 2015). Thus, BPA at a concentration of 50 mg L^{-1} was used in the presented investigation as a negative control, showing the effects of one of the building blocks which leach from PC with known adverse effects on the plants. A 27% reduction in germination percentage of *L. sativum* was observed with exposure to BPA compared to the unexposed seeds (Fig. 2, $p < 0.001$). In parallel, the solvent control, which consisted of 2% ethanol, used to dissolve the BPA, showed no significant effect on the germination after seven days ($p = 1$), showing that the germination inhibition observed can solely be attributed to BPA exposure.

Previously, BPA was shown to rapidly dissipate in soil via stable covalent bonding to the soil organic matrix forming non-extractable residues within three days and was immobilized (Fent et al., 2003). The bonding depends on the concentration of the organic matter in the soil and the particle grain size (Sun et al., 2012). The fate and bioavailability of the non-extractable residue have not been fully uncovered. Additionally, microorganisms in the soil can metabolize BPA within three days (Fent et al., 2003). In the present experiment, the cress was exposed to 4166 μg BPA kg^{-1} soil. Considering the results reported by Fent et al. (2003), who reported that approximately 85% of the BPA was unextractable after three days, 624 μg BPA kg^{-1} soil was bioavailable to the plants. Despite the rapid biodegradation of BPA in soil (West et al., 2001), soils treated with sewage sludge contained BPA concentrations of up to 150 μg kg^{-1} (Kinney et al., 2008; Langdon et al., 2012; Corrales et al., 2015). Even though the BPA concentration applied in this study (4166 μg BPA kg^{-1} soil) was much higher, the inhibition caused by new and 40-day aged PC was significantly higher, highlighting the synergistic effects of other toxic compounds leaching from the MP granules.

3.3. Seedling growth

To evaluate the effects of the artificially aged PC on *L. sativum* seedlings' growth, the root and the shoot lengths were measured after seven days of exposure in the soil containing PC, which was either new or aged for the specified times ranging from 40 to 160 days, as well as BPA. Root and shoot growth are well-established parameters (Walter and Schurr, 2005) used in the classification of xenobiotic tolerance, e.g. heavy metal tolerance of plants (Diwan et al., 2010).

In the present study, the root length (Fig. 3) of the *L. sativum* seedlings exposed to new PC granulates was significantly reduced by 40% ($p < 0.001$). Similarly, reduced root lengths in plants exposed to the 40-day and 80-day aged PC granulate were measuring equating to 37% ($p < 0.001$) and 15% ($p < 0.001$) reduction compared to the untreated control, respectively. With longer ageing of the plastic granulate, the inhibitory effect of the PC on the root growth was reduced to only 3% with 120-day aged PC granulate ($p = 1$) and using the 160-day aged PC, no significant difference in comparison to the untreated control could be measured ($p = 1$).

The shoot length followed the same pattern as displayed by the roots (Fig. 3), i.e. significantly reduced shoot length with exposures to new PC (35%; $p < 0.001$), 40-day aged PC (40%; $p < 0.001$), and 80-days aged PC (19%; $p < 0.001$) compared to the untreated control were observed. With longer ageing of the PC, the phytotoxic effect visible lowered. In exposures with 120-day ($p = 0.246$) and 160-day ($p = 1$) aged PC, the shoot lengths were not significantly reduced. Exposure to BPA in the soil led to a 34% reduction of the shoot length compared to both the control and solvent control ($p < 0.001$). The overall seedling length followed the trends displayed by the roots and the shoots. Plant seedlings remained smaller with exposure to PC new ($p < 0.001$), 40-day ($p < 0.001$), and 80-day ($p < 0.001$) aged PC and exposures to BPA ($p < 0.001$) compared to the controls.

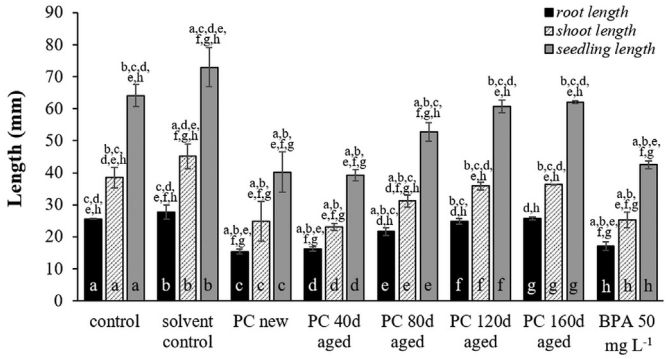

Fig. 3. Effects of new and artificially aged (in days, d) polycarbonate (PC) as well as bisphenol-A (BPA) on the root, shoot, and seedling length. Data represent the average length in mm ± standard deviation ($n = 150$). The Kruskal-Wallis test was performed (Root: $H(7) = 825.6$, $p < 0.001$; Shoot: $H(7) = 797.4$, $p < 0.001$; Seedling: $H(7) = 852.5$, $p < 0.001$) followed by pair-wise comparison. Statistical significance ($p < 0.05$) between groups is represented by the letters above the bars; i.e. a: compared to control; b: compare to solvent control; c: compared to PC new; d: compared to 40d; e: compared to 80d; f: 120d, g: 160d; and h: BPA.

In addition to the mechanisms on how MP might influence plants in terrestrial systems (Rillig et al., 2019), it is hypothesised that leaching of additives might also be one of the mechanisms, if not the most important one, at least for new MP distributed in the environment (Suhrhoff and Scholz-Böttcher, 2016). The reduced growth rate observed here may be attributed to endocrine-disrupting substances leaching from the PC, as previously elucidated with Pathway-Based Analysis (PBA) (Wang et al., 2015). The reduced inhibitory effect observed with extended ageing is proposed to occur because most of the toxic substances may already have leached from the plastic with time during the ageing process.

It was suggested that MP influence the bulk density of soil and even improve soil aeration (De Souza Machado et al., 2018), which, hypothetically, can be beneficial for root growth. However, the research presented showed that root growth is negatively affected by PC particles in a short-term experiment of seven days. Other parameters such as shoot length followed the same pattern, being similarly adversely affected by the PC exposures.

The weight characteristics (fresh weight (FW) and dry weight (DW)) of *L. sativum* seedlings (Table 1) showed that exposure to new PC, 40-day aged, 80-day aged PC granulate and BPA, adversely affected root weight significantly ($p < 0.05$). The reduction ranged from 13% to 38% for the FW and 12% to 41% for the DW of seedling roots. PC that was artificially aged longer did not result in reduced root weight ($p > 0.05$). Shoot weights and the overall seedling lengths (FW and DW) followed the same pattern.

During seedling development, the FW and DW continuously change. However, the overall reduction of plant growth and weight is reported as a stress marker mainly in drought stress situations or under salt stress (Delgado et al., 1994; Taïbi et al., 2013) and correlates to the inhibition of cell elongation (Bandeoglu et al., 2004).

Decreased fresh and dry weights of seedlings are known stress-indicators (Lichtenthaler, 1996; Tiwari et al., 2017), signifying that the seedlings experienced physiological stress. Thus, investigations into how the pigment status and antioxidative enzyme catalase were affected were investigated.

3.4. Pigments

Photosynthetic pigments play a vital role in maintaining plant vitality by playing a central role in the photosynthetic process through light absorption and excitation energy transfer towards the reaction centre (Viljevac et al., 2013). The chlorophyll *a* and *b* concentrations (Fig. 4) in the *L. sativum* seedlings' leaves were measured, as changes in

Table 1
Root, shoot, and total seedling fresh and dry weight (in mg) of *L. sativum* seedlings exposed to new and different aged (in days, d) polycarbonate (PC). Data represent mean weight ± standard deviation (SD) (n = 150). The Kruskal-Wallis test was performed (FW root: H(7) = 793.7, p < 0.001; DW root: H(7) = 814.7, p < 0.001; FW shoot: H(7) = 895.8, p < 0.001; DW shoot: H(7) = 812.5, p < 0.001; FW seedling: H(7) = 898.2, p < 0.001; DW seedling: H(7) = 833.8, p < 0.001) followed by pair-wise comparison. Asterisks (*) present a statistical difference compared to the control (p < 0.05).

	FW root [mg]	DW root [mg]	FW shoot [mg]	DW shoot [mg]	FW seedling [mg]	DW seedling [mg]
Control	0.84 ± 0.01	0.08 ± 0.000	9.48 ± 0.41	0.94 ± 0.04	10.32 ± 0.41	1.02 ± 0.04
Solvent control	0.92 ± 0.08	0.09 ± 0.007	9.96 ± 0.88	0.91 ± 0.03	10.87 ± 0.95	1.00 ± 0.04
PC new	0.52 ± 0.03*	0.05 ± 0.004*	5.30 ± 1.60*	0.62 ± 0.21*	5.82 ± 1.60*	0.66 ± 0.21*
PC 40d aged	0.55 ± 0.02*	0.05 ± 0.004*	4.51 ± 0.19*	0.49 ± 0.02*	5.06 ± 0.21*	0.54 ± 0.02*
PC 80d aged	0.73 ± 0.04*	0.07 ± 0.004*	5.13 ± 0.76*	0.55 ± 0.08*	5.86 ± 0.72*	0.62 ± 0.08*
PC 120d aged	0.82 ± 0.02	0.08 ± 0.002	6.92 ± 0.88*	1.03 ± 0.04*	11.17 ± 0.38*	1.11 ± 0.04*
PC 160d aged	0.82 ± 0.01	0.08 ± 0.003	9.69 ± 0.63	0.98 ± 0.06	10.51 ± 0.63	1.06 ± 0.06
BPA 50 mg L^{-1}	0.53 ± 0.05*	0.05 ± 0.006*	7.32 ± 0.81*	0.74 ± 0.08*	7.85 ± 0.77*	0.79 ± 0.08*

chlorophylls and chlorophyll ratio is a known stress marker (Lichtenthaler, 1996; Pavlovic et al., 2014; Sarker and Oba, 2018).

In *L. sativum* exposed to new PC granulate, 40-day aged PC granulate, and BPA at a 50 mg L^{-1}, the Chl *a* concentration was reduced by 58%, 49%, and 30%, respectively ($p < 0.001$). This decrease in the pigment content can be correlated to a decline in net photosynthesis (Qu et al., 2012). A similar decrease was seen in the Chl *b* content with exposure to new PC ($p < 0.001$), 80 day aged ($p < 0.001$) and 120 day aged PC ($p = 0.019$). Exposure to 40-day-aged PC resulted in the Chl *b* concentration returning to that equal to the control ($p = 1$); however, this is likely to the large deviation observed. Exposure to MP in soil produced a reduction in the ratio of Chl *a* to Chl *b*, indicating an increase in the antenna complex of PS II, and therefore an increase of absorption of photons. An excess of electrons in PS II can generate triplet chlorophyll and singlet oxygen formation leading to oxidative stress and possible damages (Durrant et al., 1990; Takagi et al., 2016). Singlet oxygen induces selective and also irreversible bleaching of chlorophylls that constitute P680 (De Las Rivas et al., 1993), leading to the photodamage of P680 in the PS II reaction centre (Telfer et al., 1987), massive damage and a severe negative influence on the energy production in the plant.

The lower Chl *a* and Chl *b* concentrations in exposures to new and 40-day aged PC are thus signs of stress and contributed to the reduced plant growth observed in terms of root and length, and weight.

3.5. Antioxidative stress: Catalase (CAT)

The catalase (CAT) activity as part of the antioxidative defence system (Fig. 5) was measured after PC exposure for seven days. Except for the 160-day aged PC, all artificially aged PC granulate exposures significantly affected the CAT activity in both the roots and shoots of the *L. sativum* seedlings. The maximum CAT activity was observed in the roots exposed to new PC granulate and 40-day aged PC with an enhancement of 260% ($p < 0.001$) and 242% ($p < 0.001$), respectively, compared to the untreated control. Exposure to 120-day aged PC enhanced the CAT activity by 77% ($p < 0.001$). However, exposure to 160 days aged PC did cause significant elevation ($p = 1$).

The measured CAT activity in the shoots matched the trend observed in the roots (Fig. 5). Maximum enhancements were recorded in treatments with new PC and 40-day aged PC, which increased by 118% ($p < 0.001$) and 93% ($p < 0.001$), respectively, compared to untreated controls (Fig. 5). Ageing for prolonged periods resulted in reduced enhancements of the CAT activity in the shoots ($p < 0.001$) compared to untreated controls, except for the treatment with 160-day aged PC ($p = 1$).

The induction of oxidative stress by MP was shown in the marine copepod *Paracyclopina nana* influencing the MAPK/Nrf2 pathway (Jeong et al., 2017). Similar to the proposed adverse outcome pathway (AOP) for MP exposure in this marine copepod (Jeong et al., 2017), we hypothesis that the mode of action starts with the generation of oxidative stress on a subcellular level increasing the expression of the antioxidative genes. The oxidative stress might lead to cell damage on a cellular level, and finally, MP accumulation in organisms might influence the growth rate and fecundity as an adverse outcome (Fig. 5).

3.6. Stress tolerance index (STI)

Stress tolerance index (STI) has been useful in identifying stress-tolerant genotypes of barley (El-Hashash and Agwa, 2018) and also

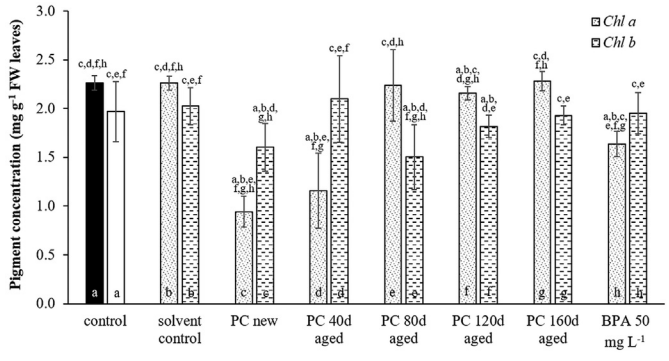

Fig. 4. Pigment content (Chl *a* and Chl *b*) in leaves of *L. sativum* exposed in soil containing polycarbonate (PC) artificially aged for varying times (in days, d) as well as bisphenol-A (BPA). The Kruskal-Wallis test was performed (Chl *a*: H(7) = 390.8, p < 0.001; Chl *b*: H(7) = 142.6, p < 0.001), followed by pair-wise comparison. Data represent average pigment concentration in mg g^{-1} ± standard deviation ($n = 50$) Statistical significance ($p < 0.05$) between groups is represented by the letters above the bars; i.e. a: compared to control; b: compare to solvent control; c: compared to PC new; d: compared to 40d; e: compared to 80d; f: 120d, g: 160d; and h: BPA.

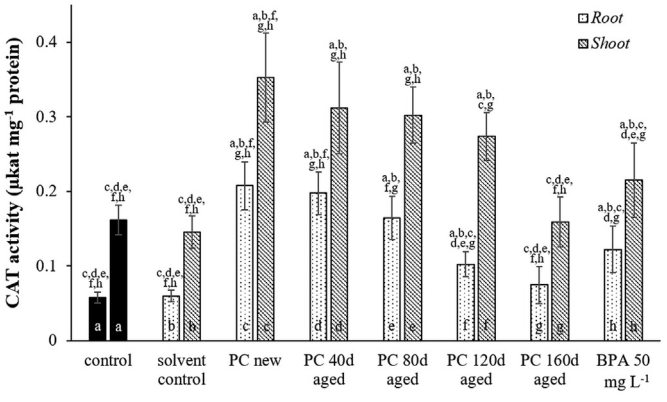

Fig. 5. Catalase activity in roots and leaves (shoots) of *L. sativum* exposed to polycarbonate (PC) aged for different periods (in days, d) as well as bisphenol-A (BPA) in soil. Data represent average enzyme activity ± standard deviation (n = 50). The Kruskal-Wallis test was performed (Root: H(7) = 419.5, p < 0.001; Shoot: H(7) = 320.8, p < 0.001), followed by pair-wise comparison. Statistical significance (p < 0.05) between groups is represented by the letters above the bars; i.e. a: compared to control; b: compare to solvent control; c: compared to PC new; d: compared to 40d; e: compared to 80d; f: 120d, g: 160d; and h: BPA.

Table 2
Toxicity Index (%) for *L. sativum* exposed for seven days to aged polycarbonate (PC) aged for various times (in days, d) in soil. RLSTI: Root length stress tolerance index; SLSTI: Shoot length stress tolerance index; RFSTI: Root fresh weight stress tolerance index; SFSTI: Shoot fresh weight stress tolerance index; RDSTI: Root dry weight stress tolerance index; SDSTI: Shoot dry weight stress tolerance index.

Stress tolerance index [%]						
	RLSTI	SLSTI	RFSTI	SFSTI	RDSTI	SDSTI
Control	0	0	0	0	0	0
Solvent control	108	117	114	110	109	105
PC new	60	65	63	62	59	56
PC 40d aged	63	60	61	66	63	48
PC 80d aged	85	81	82	87	88	54
PC 120d aged	97	93	95	98	97	73
PC 160d aged	100	94	97	98	102	102
BPA 50 mg L^{-1}	67	66	66	64	64	72

to report differences under drought stress and salt stress (Jaleel et al., 2008). In the present study, the STI for different growth parameters (Wilkins, 1957; Amin et al., 2014) were calculated to investigate the stress tolerance potential of *L. sativum* in the various exposure scenarios (Table 2). The higher the STI, the more tolerant the plant is to the stress to which it is exposed. In Table 2, the STI of *L. sativum* was the lowest with exposure to new PC and 40-day aged PC, followed by BPA. Using the longer aged PC for exposure, the STI values increased, showing an apparent increased resilience or tolerance towards stress or, from the aged PC point of view, reduced phytotoxicity.

4. Conclusion

In the present study, the results indicated that the factor time plays a vital role in plastic material phytotoxicity as the most severe adverse effects were observed with new PC and short-term aged plastic (up to 80 days). The longer the material was aged, the lesser the harmful effects observed in *L. sativum*. Of course, the material's overall toxicity in the environment will remain the same; nonetheless, the most toxic effects can be seen at the beginning of decomposition in the environment, possibly, when toxic additives leach out. The age of plastics, i.e. the time exposed to the elements allowing transformation, breakdown, and leaching, is an essential factor in the hazard it poses to the environment and organisms. However, older plastics, which have been in the environment for long periods, do not pose a lesser threat. It is essential to investigate which compounds leach from plastics with time to entirely evaluate and understand the risk posed.

CRediT authorship contribution statement

Stephan Pflugmacher: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing. **Saila Tallinen:** Data curation, Investigation, Writing – review & editing. **Young Jun Kim:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Sanghun Kim:** Conceptualization, Investigation, Methodology, Writing – review & editing. **Maranda Esterhuizen:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Aebi, H., 1984. Catalase in vitro. Methods Enzymol. 105, 121–126.
Amin, H., Arain, B.A., Amin, F., Surhio, M.A., 2014. Analysis of growth response and tolerance index of *Glycine max* (L.) Merr. under hexavalent chromium stress. Int. J. Adv. Life Sci. 1, 231–241.
Araújo, A.P.C., Malafaia, G., 2021. Microplastic ingestion induces behavioral disorders in mice: a preliminary study on the trophic transfer effects via tadpoles and fish. J. Hazard. Mater. 401, 123263. https://doi.org/10.1016/j.jhazmat.2020.123263.
Araújo, A.P.C., Emmanuela, J., Vieira, A., Malafaia, G., 2020. Toxicity and trophic transfer of polyethylene microplastics from *Poecilia reticulata* to *Danio rerio*. Sci. Total Environ. 742, 140217. https://doi.org/10.1016/j.scitotenv.2020.140217.
Bandeoglu, E., Eyidogan, F., Yucel, M., Oktem, H.A., 2004. Antioxidant responses of shoots and roots of lentil to NaCl-salinity stress. Plant Growth Regul. 42, 69–77. https://doi.org/10.1023/B:GROW.0000014891.35427.7b.
Bosker, T., Bouwman, L.J., Brun, N.R., Behrens, P., Vijver, M.G., 2019. Microplastics accumulate on pores in seed capsule and delay germination and root growth of the terrestrial vascular plant *Lepidium sativum*. Chemosphere 226, 774–781. https://doi.org/10.1016/j.chemosphere.2019.03.163.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.
Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). Environ. Sci. Technol. 42, 5026–5031. https://doi.org/10.1021/es800249a.
Bush, D.S., 1995. Calcium regulation in plant cells and its role in signaling. Annu. Rev. Plant Biol. 46, 95–122. https://doi.org/10.1146/annurev.pp.46.060195.000523.
Carwile, J.L., Luu, H.T., Bassett, L.S., Driscoll, D.A., Yuan, C., Chang, J.Y., Ye, X., Calafat, A.M., Michels, K.B., 2009. Polycarbonate bottle use and urinary bisphenol A concentrations. Environ. Health Perspect. 117 (9), 1368–1372. https://doi.org/10.1289/ehp.0900604.
Chapin, R.E., Adams, J., Boekelheide, K., Gray Jr., L.E., Hayward, S.W., Lees, P.S., et al., 2008. NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. Birth Defects Res. B Dev. Reprod. Toxicol. 83, 157–395. https://doi.org/10.1002/bdrb.20147.
Chou, C.-H., Lin, H.-J., 1976. Autointoxication mechanism of *Oryza sativa* I. Phytotoxic effects of decomposing rice residues in soil. J. Chem. Ecol. 2, 353–367. https://doi.org/10.1007/BF00988282.
Choy, C.A., Robison, B.H., Gagne, T.O., et al., 2019. The vertical distribution and biological transport of marine microplastics across the epipelagic and mesopelagic water column. Sci. Rep. 9, 7843. https://doi.org/10.1038/s41598-019-44117-2.
Cole, M., Lindeque, P., Filemann, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T.S., 2013. Microplastic ingestion by zooplankton. Environ. Sci. Technol. 47, 6646–6655. https://doi.org/10.1021/es400663f.
Corrales, J., Kristofco, L.A., Steele, W.B., Yates, B.S., Breed, C.S., Williams, E.S., Brooks, B.W., 2015. Global assessment of bisphenol A in the environment: review and analysis of its occurrence and bioaccumulation. Dose-Response 13 (3), 1559325815598308. https://doi.org/10.1177/1559325815598308.
Cox, K.D., Covernton, G.A., Davies, H.L., Dower, J.F., 2019. Human consumption of microplastics. Environ. Sci. Technol. 53, 7068–7074. https://doi.org/10.1021/acs.est.9b01517.
Day, R.H., 1986. Report on the cruise of the Pusan 851 to the North Pacific Ocean, July–August 1986. Final Report to National Oceanic and Atmospheric Administration. National Marine Fisheries Service, Auke Bay Laboratory, Auke Bay, AK (93 pp.).
De Las Rivas, J., Shipton, C.A., Ponticos, M., Barber, J., 1993. Acceptor side mechanism of photoinduced proteolysis of the D1 protein in photosystem II reaction centers. Biochem. 32, 6944–6950. https://doi.org/10.1021/bi00078a019.
De Souza Machado, A.A., Lau, C.W., Till, J., Kloas, W., Lehmann, A., Becker, R., Rillig, M.C., 2018. Impacts of microplastics on the soil biophysical environment. Environ. Sci. Technol. 52, 9656–9665. https://doi.org/10.1021/acs.est.8b02212.
Deirram, N., Rahmat, A.R., 2012. Hydrolysis degradation of polycarbonate using different co-solvent under microwave irradiation. APCBEE Procedia 3, 172–176. https://doi.org/10.1016/j.apcbee.2012.06.065.
Delgado, M.J., Ligero, F., Lluch, C., 1994. Effects of salt stress on growth and nitrogen fixation by pea, faba-bean, common bean, and soybean plants. Soil Biol. Biochem. 26, 371–376. https://doi.org/10.1016/0038-0717(94)90286-0.
Desforges, J.P., Galbraith, M., Ross, P.S., 2015. Ingestion of microplastics by zooplankton in the northeast Pacific Ocean. Arch. Environ. Contam. Toxicol. 69, 320–330. https://doi.org/10.1007/s00244-015-0172-5.
Diwan, H., Khan, I., Ahmad, A., Iqbal, M., 2010. Induction of phytochelatins and antioxidant defence system in *Brassica juncea* and *Vigna radiata* in response to chromium treatments. Plant Growth Regul. 61, 97–107. https://doi.org/10.1007/s10725-010-9454-0.
Durrant, J.R., Giorgi, L.B., Barber, J., Klug, D.R., Porter, G., 1990. Characterisation of triplet states in isolated photosystem II reaction centers: oxygen quenching as a mechanism for photodamage. Biochim. Biophys. Acta 1017, 167–175. https://doi.org/10.1016/0005-2728(90)90148-W.

El-Hashash, E.F., Agwa, A.M., 2018. Genetic parameters and stress tolerance index for quantitative traits in barley under different drought stress severities. Asian J. Res. Crop Sci. 1, 1–16. https://doi.org/10.9734/AJRCS/2018/38702.
Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ. Pollut. 177, 1–3. https://doi.org/10.1016/j.envpol.2013.01.046.
Fejdyś, M., Landwijt, M., Strusczyk, M.H., 2011. Effects of accelerated ageing conditions on the degradation process of dyneema® polyethylene composites. Fibres Text. East. Eur. 19, 60–65.
Fent, G., Hein, W.J., Moendel, M.J., Kubiak, R., 2003. Fate of 14C-bisphenol A in soils. Chemosphere 51, 735–746. https://doi.org/10.1016/S0045-6535(03)00100-0.
Fuller, S., Gautam, A.A., 2016. Procedure for measuring microplastics using pressurised fluid extraction. Environ. Sci. Technol. 50 (11), 5774–5780. https://doi.org/10.1021/acs.est.6b00816.
Gray, A.D., Weinstein, J.E., 2017. Size- and shape-dependent effects of microplastic particles on adult daggerblade grass shrimp. Environ. Toxicol. Chem. 36, 3074–3080. https://doi.org/10.1002/etc.3881.
Hamid, S.H., Amin, M.B., Maadhah, A.G., 1992. Handbook of Polymer Degradation. Marcel Decker, New York.
He, D., Luo, Y., Lu, S., Liu, M., Song, Y., Lei, L., 2018. Microplastics in soils: analytical methods, pollution characteristics and ecological risks. TrAC Trends Anal. Chem. 109, 163–172. https://doi.org/10.1016/j.trac.2018.10.006.
Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future priorities. Sci. Total Environ. 586, 127–141. https://doi.org/10.1016/j.scitotenv.2017.01.190.
Inskeep, W.P., Bloom, P.R., 1985. Extinction coefficient of chlorophyll a and b in N,N-dimethylformamide and 80% acetone. Plant Physiol. 77, 483–485. https://doi.org/10.1104/pp.77.2.48.
International Organization for Standardization (ISO), 1996. Water quality - Determination of the acute lethal toxicity of substances to a freshwater fish [Brachydanio rerio Hamilton-Buchanan (Teleostei, Cyprinidae)]. ISO 7346-3: Flow-through method. ISO: Paris [https://www.iso.org/standard/14030.html] (Accessed 15 May 2020).
Isobe, A., Iwasaki, S., Uchida, K., Tokai, T., 2019. Abundance of non-conservative microplastics in the upper ocean from 1957 to 2066. Nat. Commun. 10, 417–430. https://doi.org/10.1038/s41467-019-08316-9.
Jaleel, C.A., Sankar, B., Murali, P.V., Gomathinayagam, M., Lakshmanan, G.M.A., Panneerselvam, R., 2008. Water deficit stress effects on reactive oxygen metabolism in *Catharanthus roseus*: impacts on ajmalicine accumulation. Colloids Surf. B: Biointerfaces 62, 05–111. https://doi.org/10.1016/j.colsurfb.2007.09.026.
Janssen, J.G.M., 1973. A method of recording germination curves. Ann. Bot. 37, 705–708.
Jeong, C.-B., Kang, H.-M., Lee, M.-C., Kim, D.-H., Han, J., Hwang, D.-S., Souissi, S., Lee, S.-J., Shin, K.-H., Park, H.G., Lee, J.-S., 2017. Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepode Paracyclopina nana. Nat. Sci. Rep. 7, 41323–41334. https://doi.org/10.1038/srep41323.
Kinney, C.A., Furlong, E.T., Kolpin, D.W., Burkhardt, M.R., Zaugg, S.D., Werner, S.L., Bossio, J.P., Benotti, M.J., 2008. Bioaccumulation of pharmaceuticals and other anthropogenic waste indicators in earthworms from agricultural soil amended with biosolid or swine manure. Environ. Sci. Technol. 42 (6), 1863–1870. https://doi.org/10.1021/es702304c.
Koelmans, A.A., 2015. Modeling the role of microplastics in bioaccumulation of organic chemicals to marine aquatic organisms. A critical review. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer, Berlin, pp. 313–328.
Langdon, K.A., Warne, M.S.J., Smernik, R.J., Shareef, A., Kookana, R.S., 2012. Field dissipation of 4-nonylphenol, 4-t-octylphenol, triclosan and bisphenol A following land application of biosolids. Chemosphere 86, 1050–1058. https://doi.org/10.1016/j.chemosphere.2011.11.057.
Lee, S.-N., Stolarski, V., Letton, A., Laane, J., 2000. Studies of bisphenol-A–polycarbonate aging by Raman difference spectroscopy☆In honour of Professor Giuseppe Zerbi on the occasion of his 65th birthday.☆. J. Mol. Struct. 521, 19–24. https://doi.org/10.1016/S0022-2860(99)00422-6.
Liang, C.C., Lin, C.H., Cheng, T.C., Shieh, J., Lin, H.H., 2015. Nanoimprinting of flexible polycarbonate sheets with a flexible polymer mold and application to superhydrophobic surfaces. Adv. Mater. Interfaces 2, 1500030. https://doi.org/10.1002/admi.201500030.
Lichtenthaler, H.K., 1996. Vegetation stress: an introduction to the stress concept in plants. J. Plant Physiol. 148, 4–14. https://doi.org/10.1016/S0176-1617(96)80287-2.
Lu, Y.F., Zhang, Y., Deng, Y., Jiang, W., Zhao, Y., Geng, J., Ding, L., Ren, H., 2016. Uptake and accumulation of polystyrene microplastics in zebrafish (*Danio rerio*) and toxic effects in liver. Environ. Sci. Technol. 50, 4054–4060. https://doi.org/10.1021/acs.est.6b00183.
Meixner, K., Kubiczek, M., Fritz, I., 2020. Microplastic in Soil–Current Status in Europe With Special Focus on Method Tests With Austrian Samples.
Moore, C.J., 2008. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. Environ. Res. 108, 131–139. https://doi.org/10.1016/j.envres.2008.07.025.
Pavlovic, D., Nikolic, B., Durovic, S., Waisi, H., Andelkovic, A., Marisavljevic, D., 2014. Chlorophyll as a measure of plant health: agroecological aspects. Pestic. Phytomed. 29, 21–34. https://doi.org/10.2298/PIF1401021P.
Pflugmacher, S., Sulek, A., Mader, H., Heo, J., Noh, J.H., Penttinen, O.-P., Kim, Y., Kim, S., Esterhuizen, M., 2020. The influence of new and artificial aged microplastic and lleachates on the germination of *Lepidium sativum* L. Plants 9, 339. https://doi.org/10.3390/plants9030339.
Piehl, S., Leibner, A., Loder, M.G.J., Dris, R., Bogner, C., Laforsch, C., 2018. Identification and quantification of macro- and microplastics on an agricultural farmland. Sci. Rep. 8, 17950. https://doi.org/10.1038/s41598-018-36172-y.
PN-EN 12280-1:2002 Rubber- or plastics-coated fabrics – accelerated ageing tests – part 1: heat ageing (EN 12280-1:1997).
Qu, C., Liu, C., Gong, X., Li, C., Hong, M., Wang, L., Hong, F., 2012. Impairment of maise seedling photosynthesis caused by a combination of potassium deficiency and salt stress. Environ. Exp. Bot. 75, 134–141. https://doi.org/10.1016/j.envexpbot.2011.08.019.
Ram, A., Zilber, O., Kenig, S., 1985. Residual stresses and toughness of polycarbonate exposed to environmental conditions. Polym. Eng. Sci. 25, 577–581. https://doi.org/10.1002/pen.760250911.
Rehse, S., Kloas, W., Zarfl, C., 2016. Short-term exposure with high concentrations of pristine microplastic particles leads to immobilisation of *Daphnia magna*. Chemosphere 153, 91–99. https://doi.org/10.1016/j.chemosphere.2016.02.133.
Rillig, M.C., 2012. Microplastic in terrestrial ecosystems and the soil? Environ. Sci. Technol. 46, 6453–6454. https://doi.org/10.1021/es302011r.
Rillig, M.C., Lehmann, A., de Souza Machado, A.A., Yang, G., 2019. Microplastic effects on plants. New Phytol. 223, 1066–1070. https://doi.org/10.1111/nph.15794.
Rochman, C.M., Brownw, M.A., Halpern, B.S., Hentschel, B.T., Hoh, E., Karapanagioti, H.K., Rios-Mendoza, L.M., Takada, H., Teh, S., Thompson, R.C., 2013. Policy: classify plastic waste as hazardous. Nature 494, 169–171. https://doi.org/10.1038/494169a.
Sarker, U., Oba, S., 2018. Augmentation of leaf colour parameters, pigments, vitamins, phenolic acids, flavonoids and antioxidative activity in selected *Amaranthus tricolor* under salinity stress. Sci. Rep. 8, 12349. https://doi.org/10.1038/s41598-018-30897-6.
Scopetani, C., Chelazzi, D., Cincinelli, A., Esterhuizen-Londt, M., 2019. Assessment of microplastic pollution: occurrence and characterisation in Vesijärvi lake and Pikku Vesijärvi pond, Finland. Environ. Monit. Assess. 191, 652. https://doi.org/10.1007/s10661-019-7843-z.
Scopetani, C., Esterhuizen-Londt, M., Chelazzi, D., Cincinelli, A., Setälä, H., Pflugmacher, S., 2020. Self-contamination from clothing in microplastics research. Ecotoxicol. Environ. Saf. 189, 110036. https://doi.org/10.1016/j.ecoenv.2019.110036.
Scott, S.J., Jones, R.A., Williams, W.A., 1984. Review of data analysis for seed germination. Crop Sci. 24, 1192–1199. https://doi.org/10.2135/cropsci1984.0011183X002400060043x.
Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185, 77–83. https://doi.org/10.1016/j.envpol.2013.10.013.
Shaw, D.G., Mapes, G.A., 1979. Surface circulation and the distribution of pelagic tar and plastic. Mar. Pollut. Bull. 10, 160–162. https://doi.org/10.1016/0025-326X(79)90421-1.
Shekhawat, N., Sharma, A., Aggarwal, S., Nair, K.G.M., 2011. Refractive index engineering in polycarbonate implanted by 100 keV N+ ions. Opt. Eng. 50 (4), 044601. https://doi.org/10.1117/1.3562325.
Sokal, R.R., Rohlf, F.J., 1997. Biometry. The Principles and Practice of Statistic in Biological, Research. WH Freeman and Company, New York.
Suhrhoff, T.J., Scholz-Böttcher, B.M., 2016. Qualitative impact of salinity, UV radiation and turbulence on leaching of organic plastic additives from four common plastics – a lab experiment. Environ. Sci. Technol. 102, 84–94. https://doi.org/10.1016/j.marpolbul.2015.11.054.
Sun, K., Jin, J., Gao, B., Zhang, Z., Wang, Z., Pan, Z., Xu, D., Zhao, Y., 2012. Sorption of 17a-ethinyl estradiol, bisphenol A and phenanthrene to different size fractions of soil and sediment. Chemosphere 88, 577–583. https://doi.org/10.1016/j.chemosphere.2012.03.034.
Taïbi, K., Taïbi, F., Belkhodja, M., 2013. Salinity effects on the physiological response of two bean genotypes *Phaseolus vulgaris* L. Arab Gulf J. Sci. Res. 31, 90–98.
Takagi, D., Takumi, S., Hashiguchi, M., Sejima, T., Miyake, C., 2016. Superoxide and singlet oxygen produced within the thylakoid membranes both cause photosystem I photoinhibition. Plant Physiol. 171, 1626–1634. https://doi.org/10.1104/pp.16.00246.
Telfer, A., Marder, J.B., Barber, J., 1987. Photosystem II reaction centers isolated from phosphorylated pea thylakoids carry phosphate on the D1 and D2 polypeptide subunits. Biochim. Biophys. Acta 893, 557–563. https://doi.org/10.1016/0005-2728(87)90107-1.
Tiwari, S., Bajpai, S., Srivastava, N., 2017. Some morphological and biochemical changes in Gram seedlings under cadmium stress. J. Bioremediat. Biodegrad. 8, 403–406.
Viljevac, M., Dugalic, K., Mihaljevic, I., Simic, D., Sudar, R., Jukovic, Z., Lepedus, H., 2013. Chlorophyll content, photosynthetic efficiency and genetic markers in two sour cherry (*Prunus cerasus* L.) genotypes under drought stress. Acta Bot. Croat. 72, 221–235. https://doi.org/10.2478/botcro-2013-0003.
Walter, A., Schurr, U., 2005. Dynamics of leaf and root growth: endogenous control *versus* environmental impact. Ann. Bot. 95c, 891–900. https://doi.org/10.1093/aob/mci103.
Wang, S., Wang, L., Hua, W., et al., 2015. Effects of bisphenol A, an environmental endocrine disruptor, on the endogenous hormones of plants. Environ. Sci. Pollut. Res. 22, 17653–17662. https://doi.org/10.1007/s11356-015-4972-y.
West, R.J., Goodwin, P.A., Klečka, G.M., 2001. Assessment of the ready biodegradability of bisphenol A. Bull. Environ. Contam. Toxicol. 67, 106–112. https://doi.org/10.1007/s001280097.
Wilkins, D., 1957. A technique for the measurement of lead tolerance in plants. Nature 180, 37–38. https://doi.org/10.1038/180037b0.
Yakushev, E., Gebruk, A., Osadchiev, A., et al., 2021. Microplastics distribution in the Eurasian Arctic is affected by Atlantic waters and Siberian rivers. Commun. Earth Environ. 2, 23. https://doi.org/10.1038/s43247-021-00091-0.
Zhang, Y., Kang, S., Allen, S., Allen, D., Gao, T., Sillanpää, M., 2020. Atmospheric microplastics: a review on current status and perspectives. Earth-Sci. Rev. 203, 103118. https://doi.org/10.1016/j.earscirev.2020.103118.
Zimmermann, L., Göttlich, S., Oehlmann, J., Wagner, M., Völker, C., 2020. What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to *Daphnia magna*. Environ. Pollut. 267, 115392. https://doi.org/10.1016/j.envpol.2020.115392.